



DOCKET NO: IBIS0038-103/IBIS-0490
Title: MASS SPECTROMETRIC METHODS FOR BIOMOLECULAR
SCREENING
Filing Date: June 27, 2003 Serial No. 10/608,354
Agent: Daniel M. Scolnick Atty Phone: 215 665-2000
Replacement Sheet 2 of 33

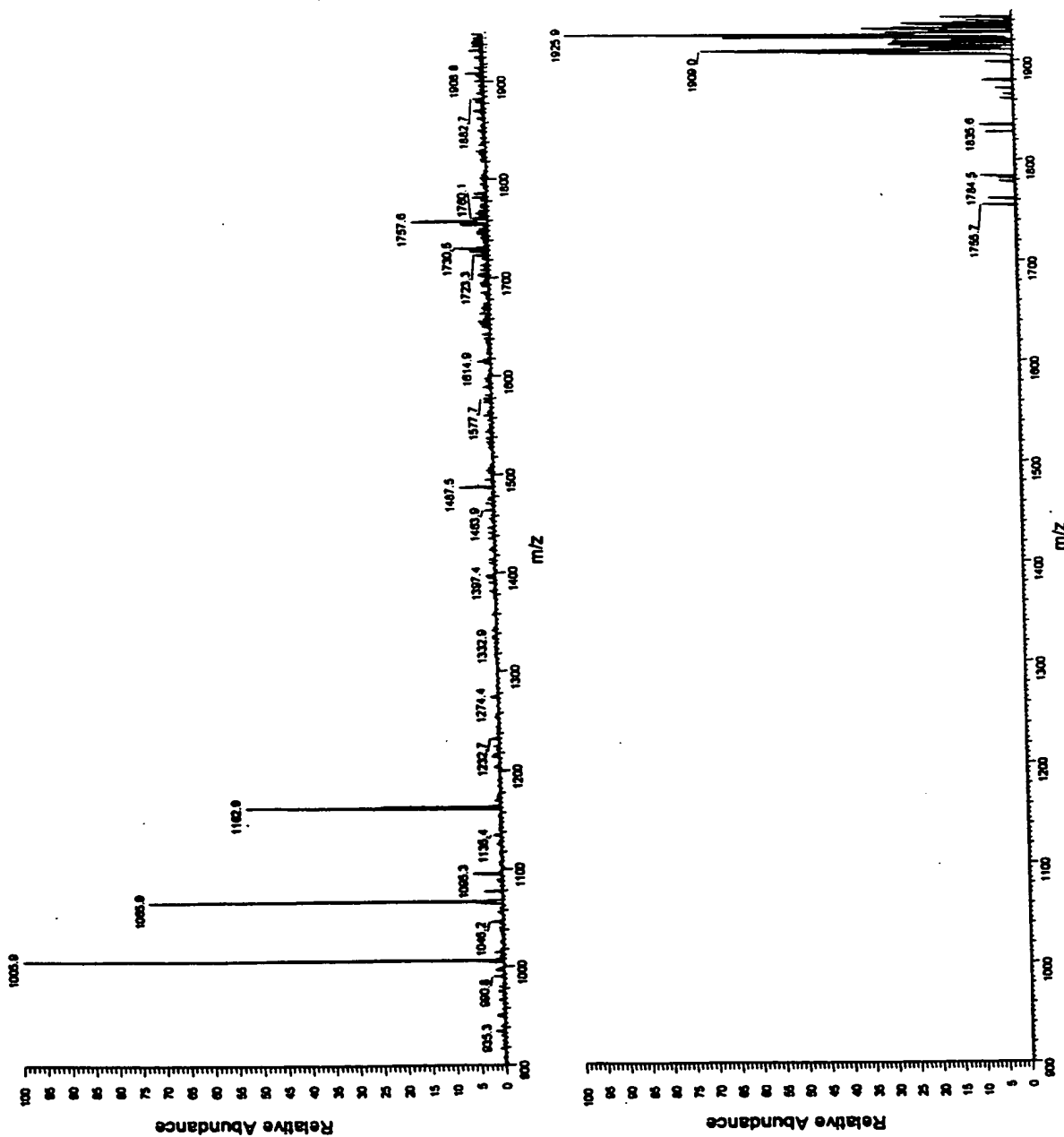


Figure 2. MS/MS of control RNA/DNA (upper); control+paromomycin (lower)

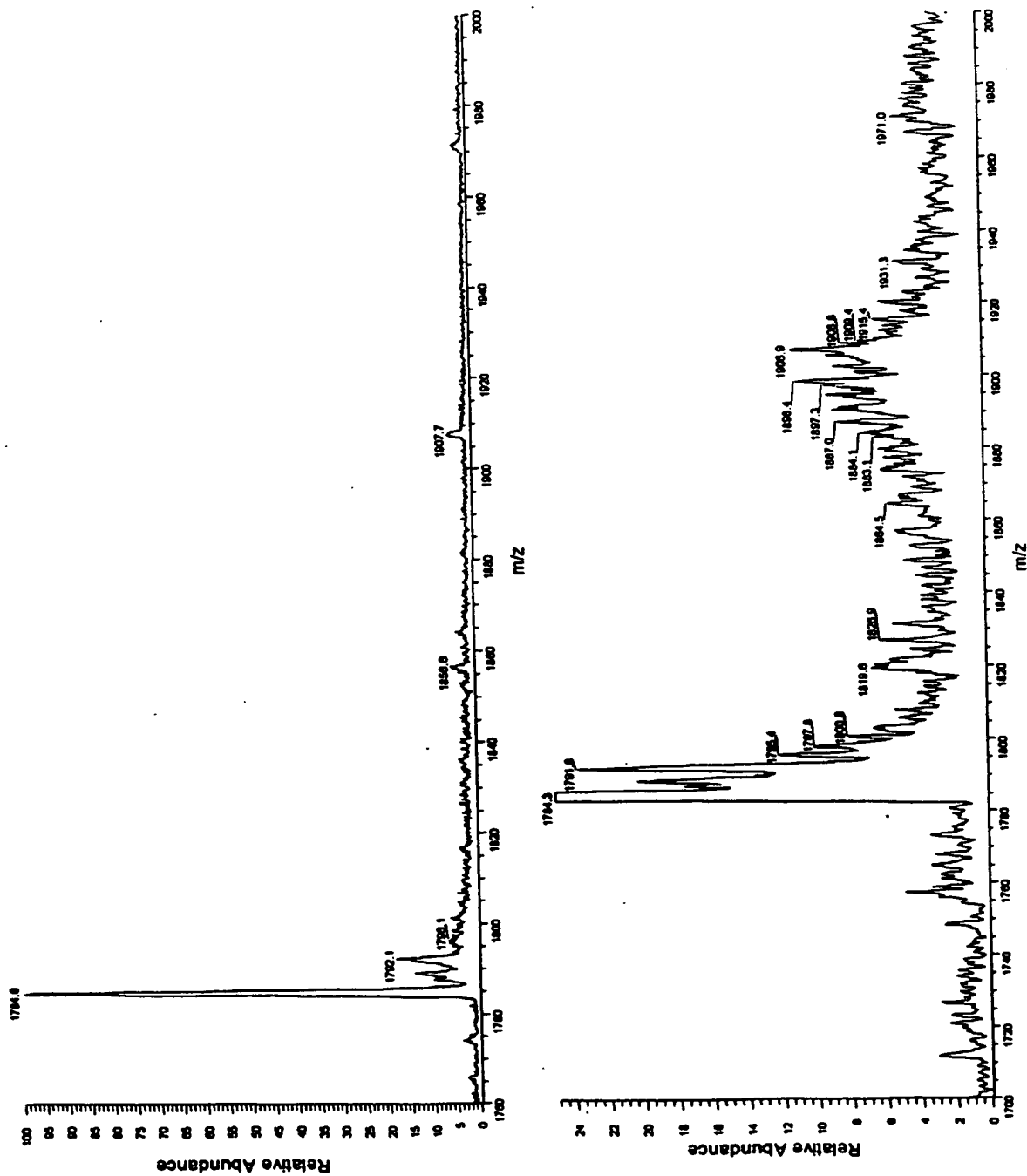


Figure 3. RNA/DNA chimera+paramomycin (upper); chimera+library (lower)

Figure 4. MS-MS analysis of member bound to RNA/DNA chimera

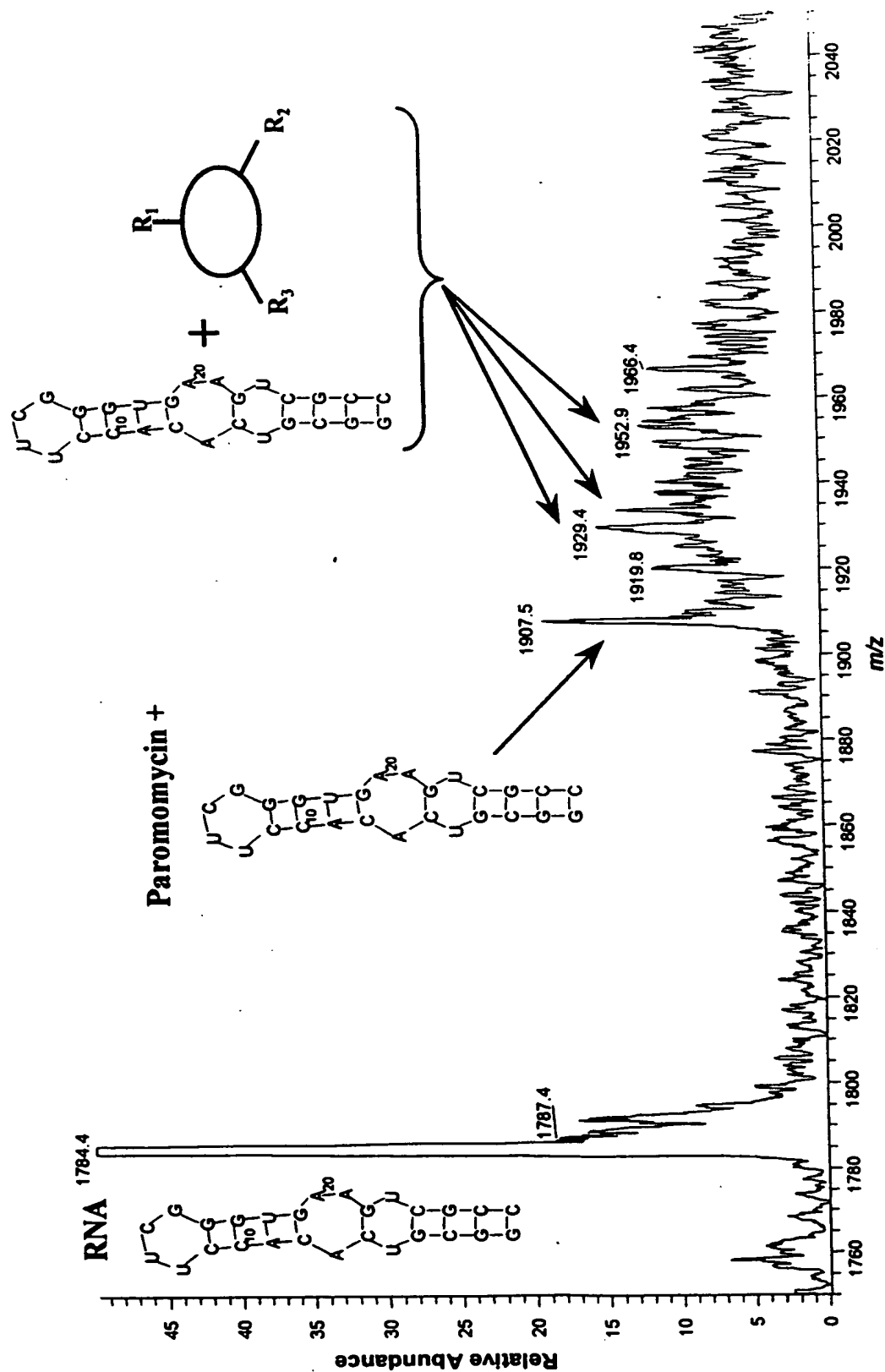


Figure 5. ESI-MS of RNA/DNA chimera bound to paromomycin and library

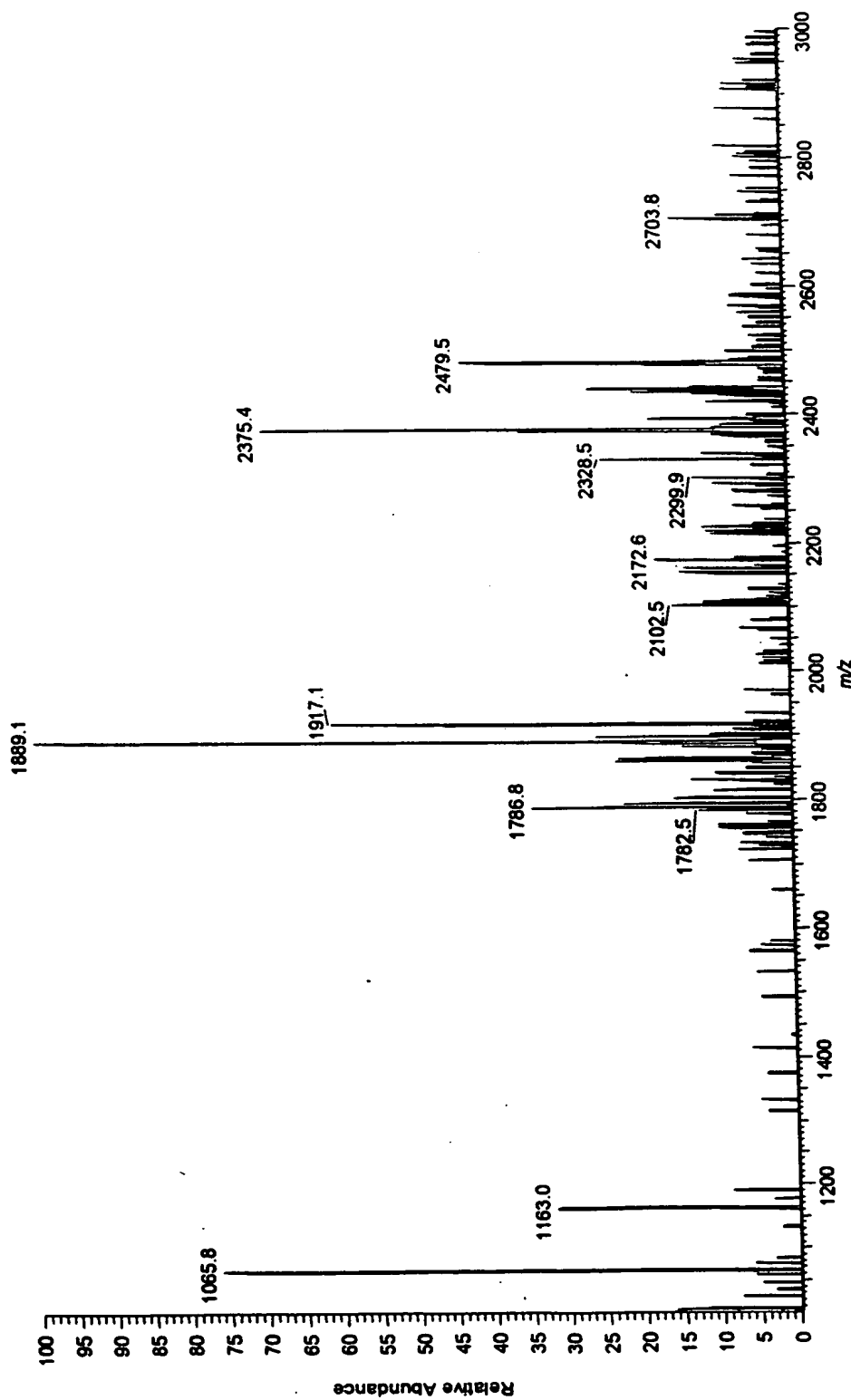


Figure 6. MS/MS of RNA/DNA chimera + compound with mass 665.1 not bound at the A-site

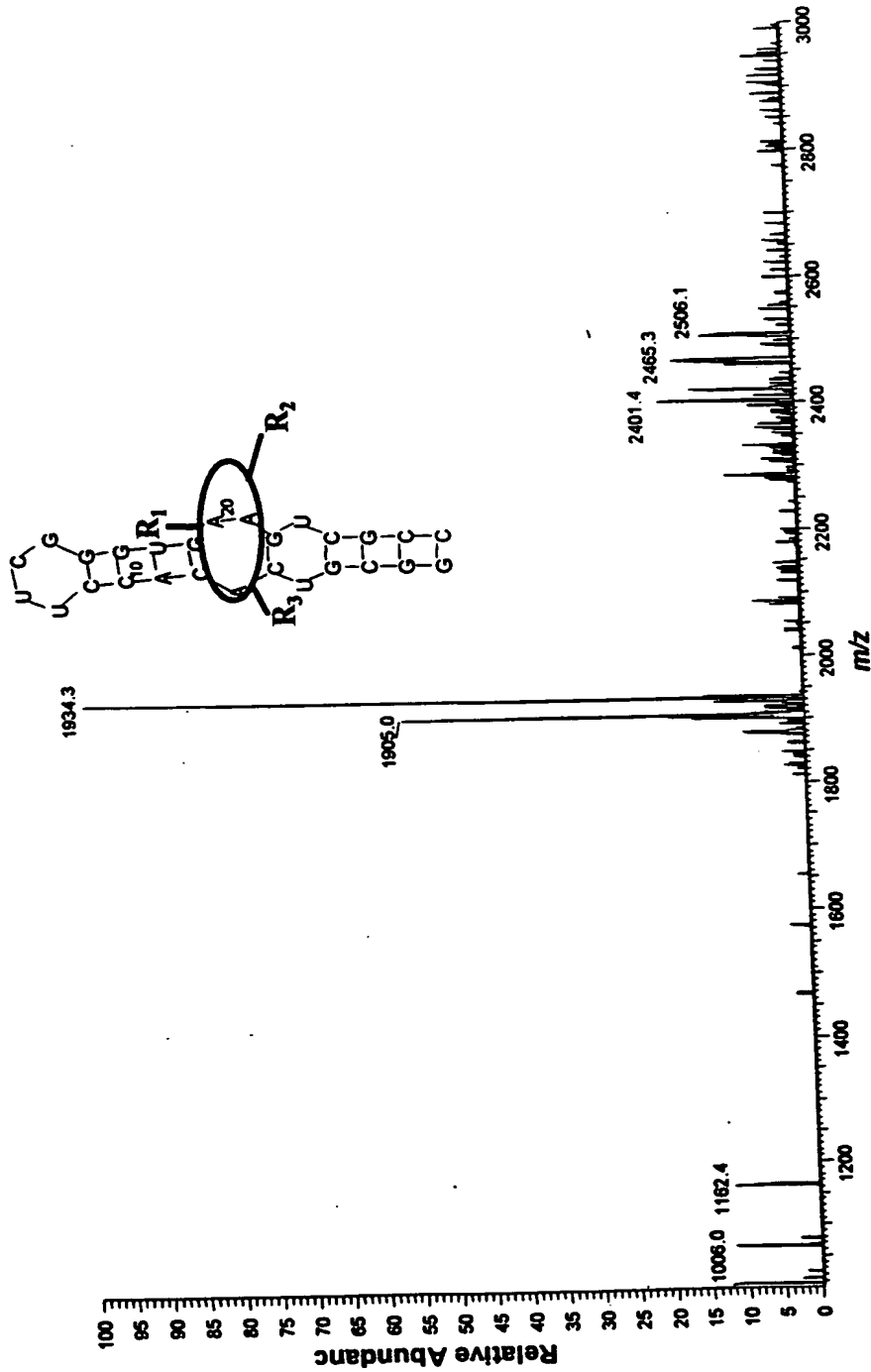
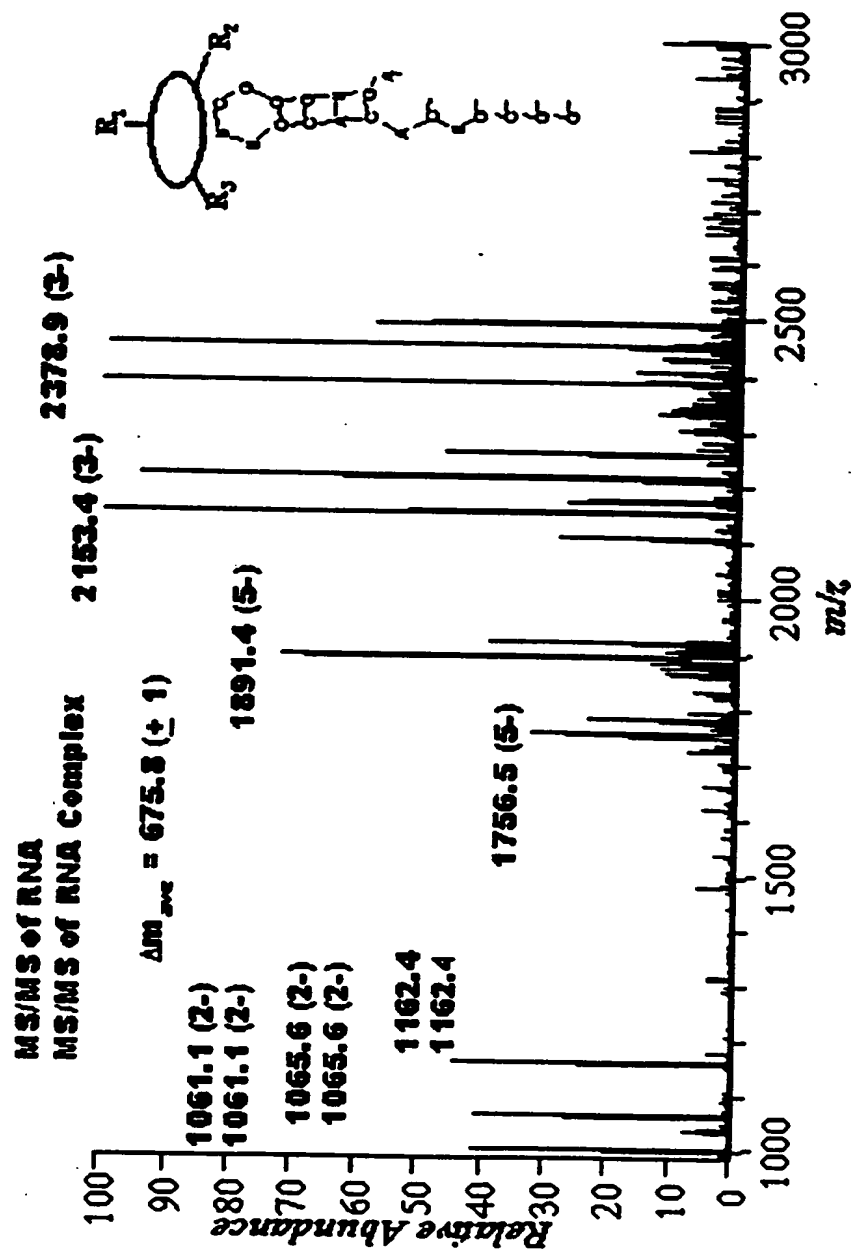


Figure 7. MS-MS analysis of member bound to RNA/DNA chimera at the A-Site

MASS Analysis of Binding Location

non-A site binder

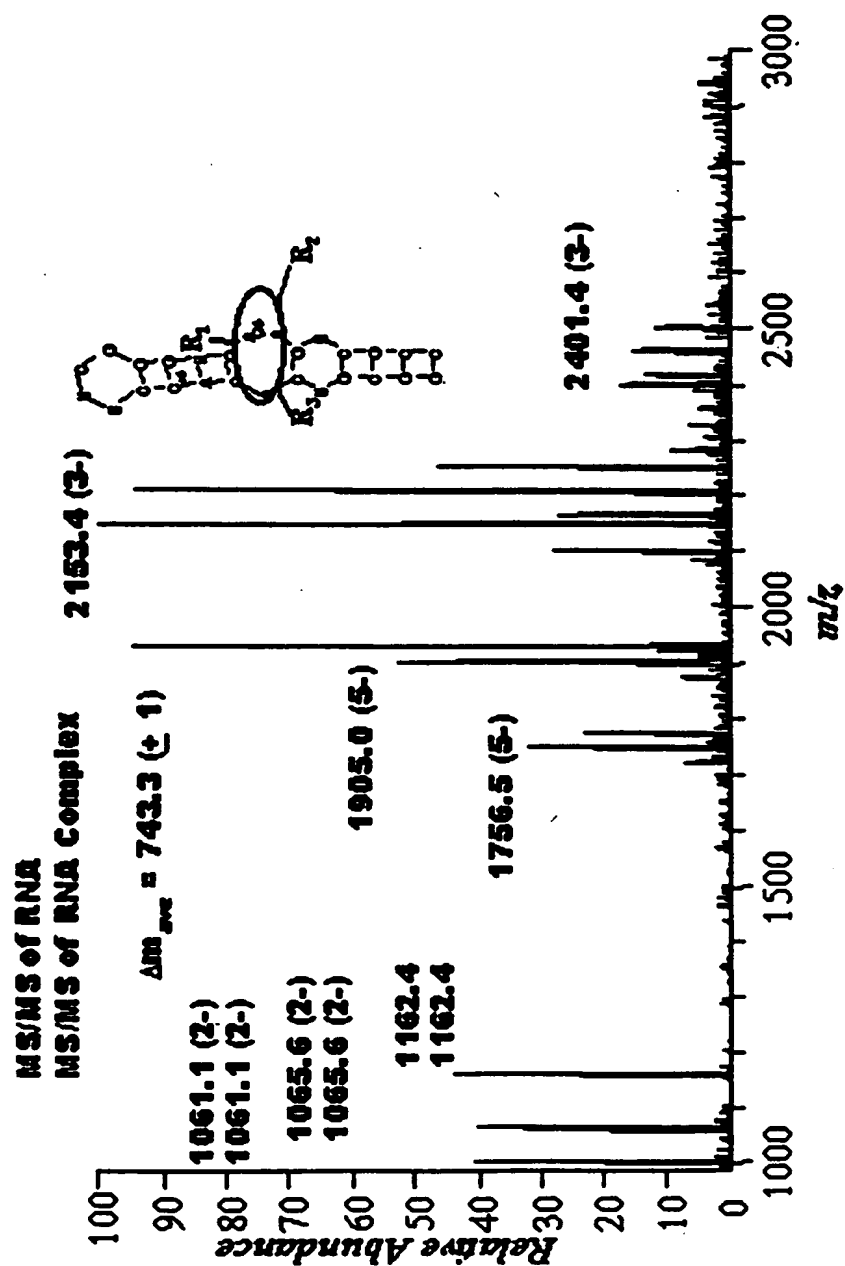
FIGURE 10



MASS Analysis of Binding Location

non-A site binder

FIGURE 11



**MASS analysis of 16S A site RNA plus
 216 member library
 (performed on quadrupole ion trap)**

FIGURE 12

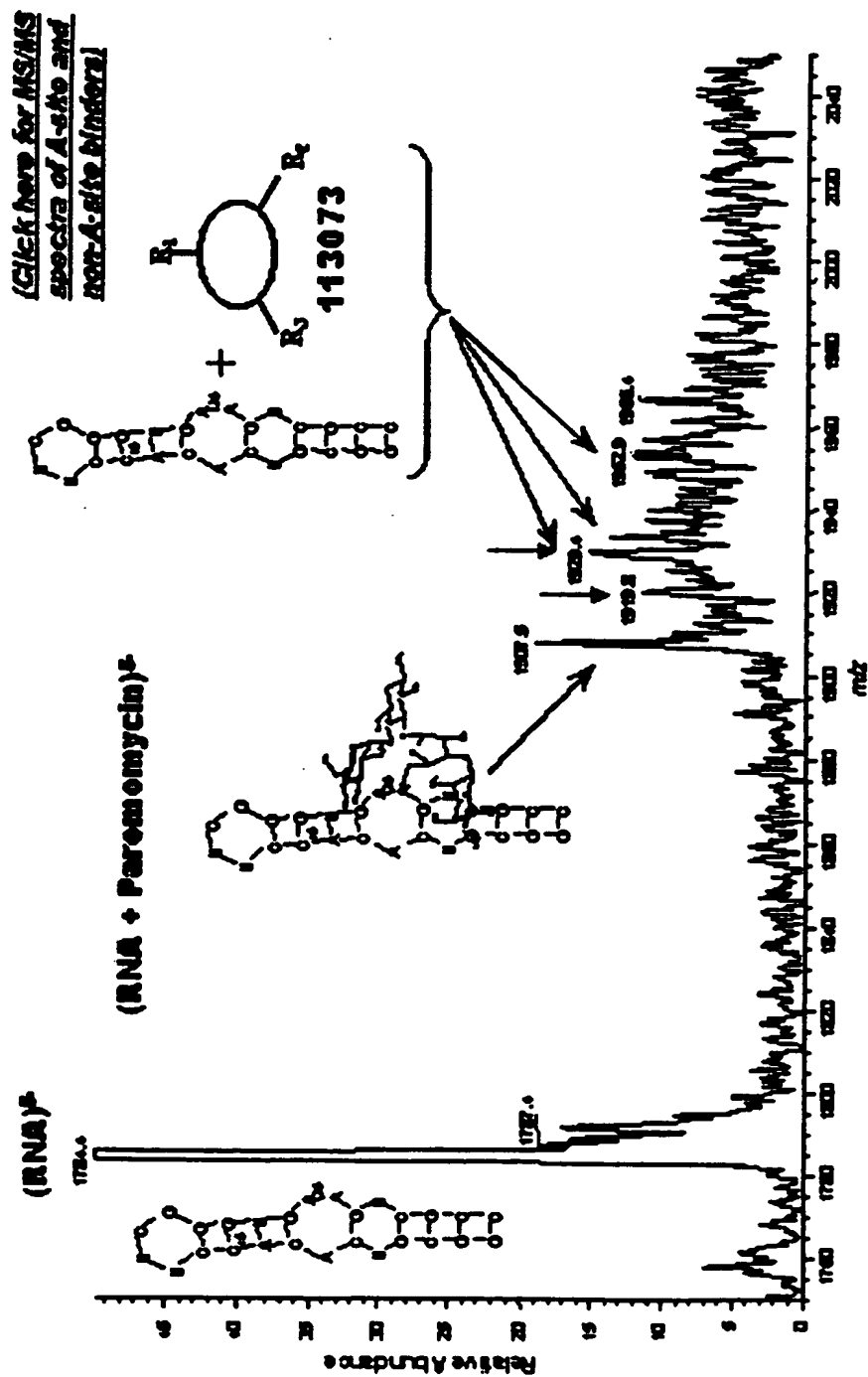


Figure 13

High Precision ESI-FTICR Mass Measurement of 16S A site RNA/Paromomycin Complex

use of unbound RNA as internal mass standard provides low ppm mass measurement errors

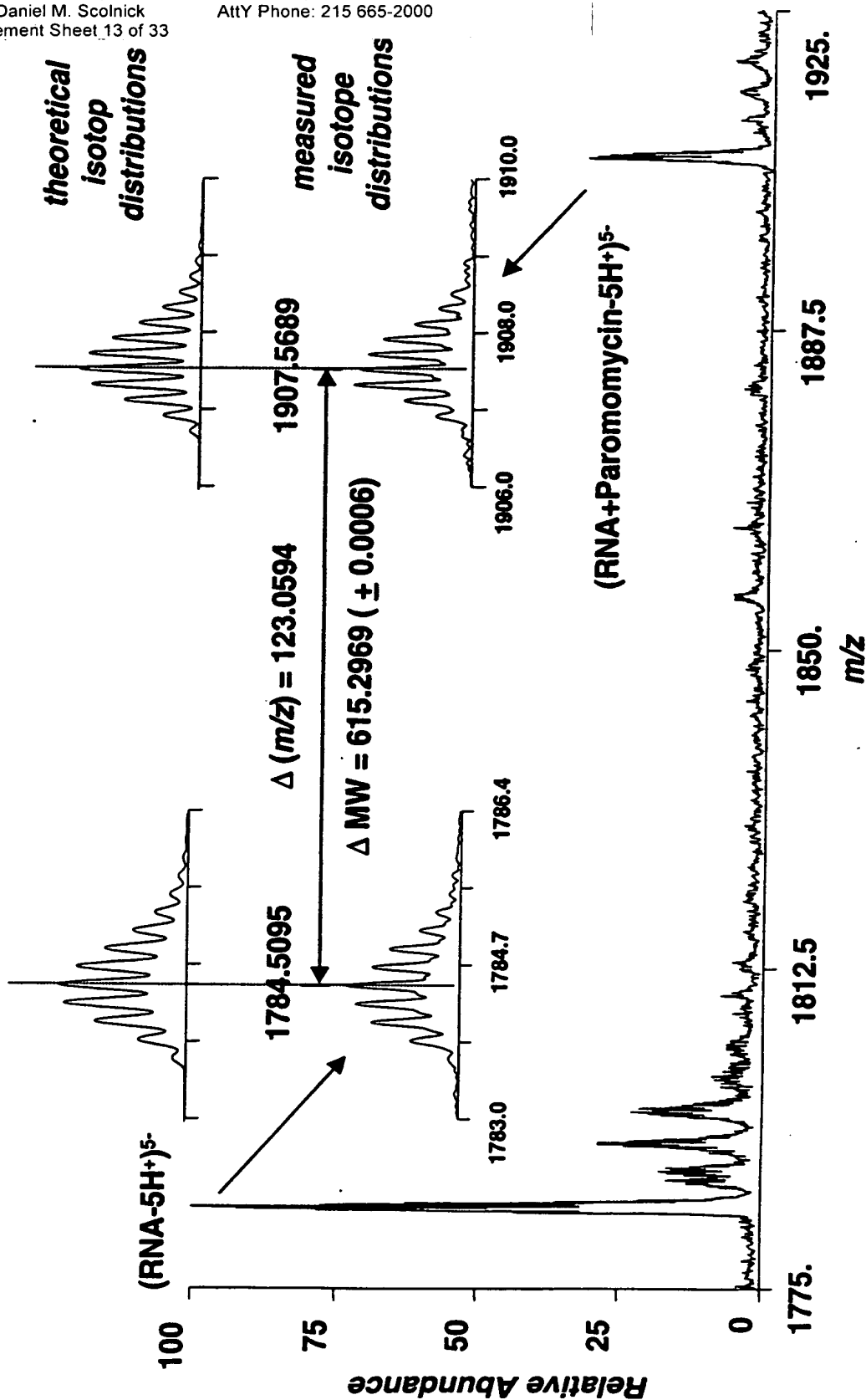
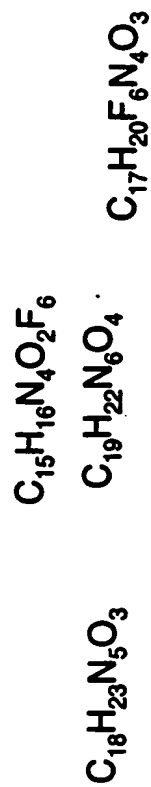


Figure 14

MASS of 60-Member Ibis Library Against 16S A-site RNA



Free 16S
RNA

Complexes

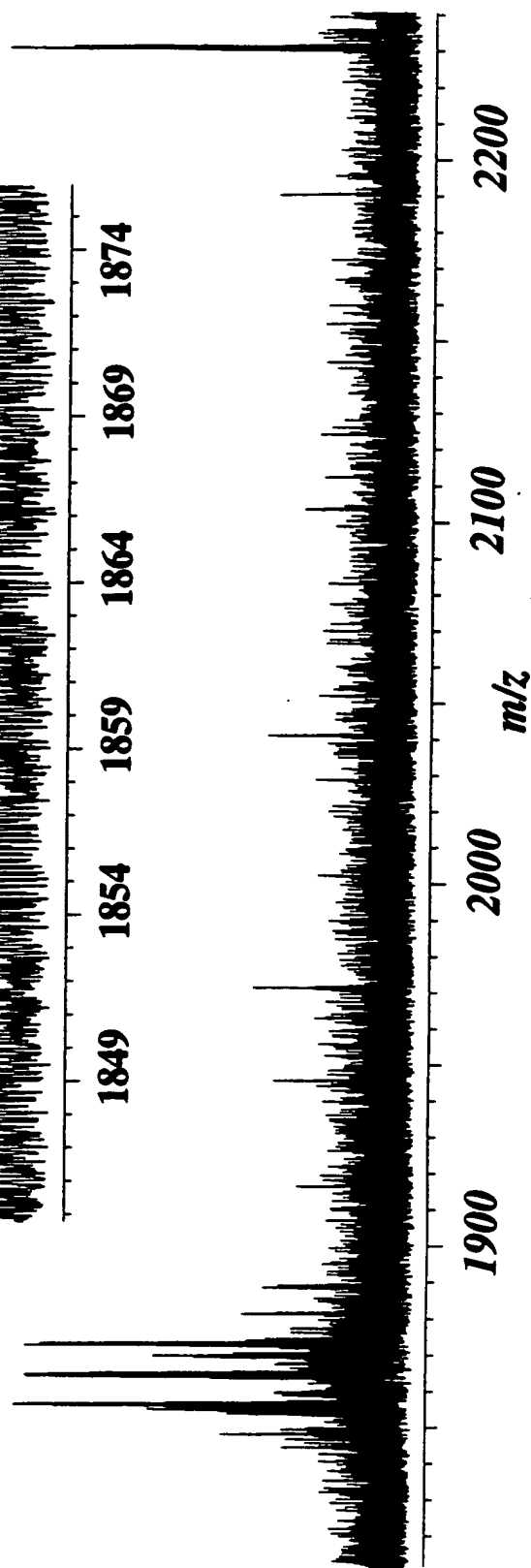


Figure 15

MASS of 60-member Library against 16S A-site Model

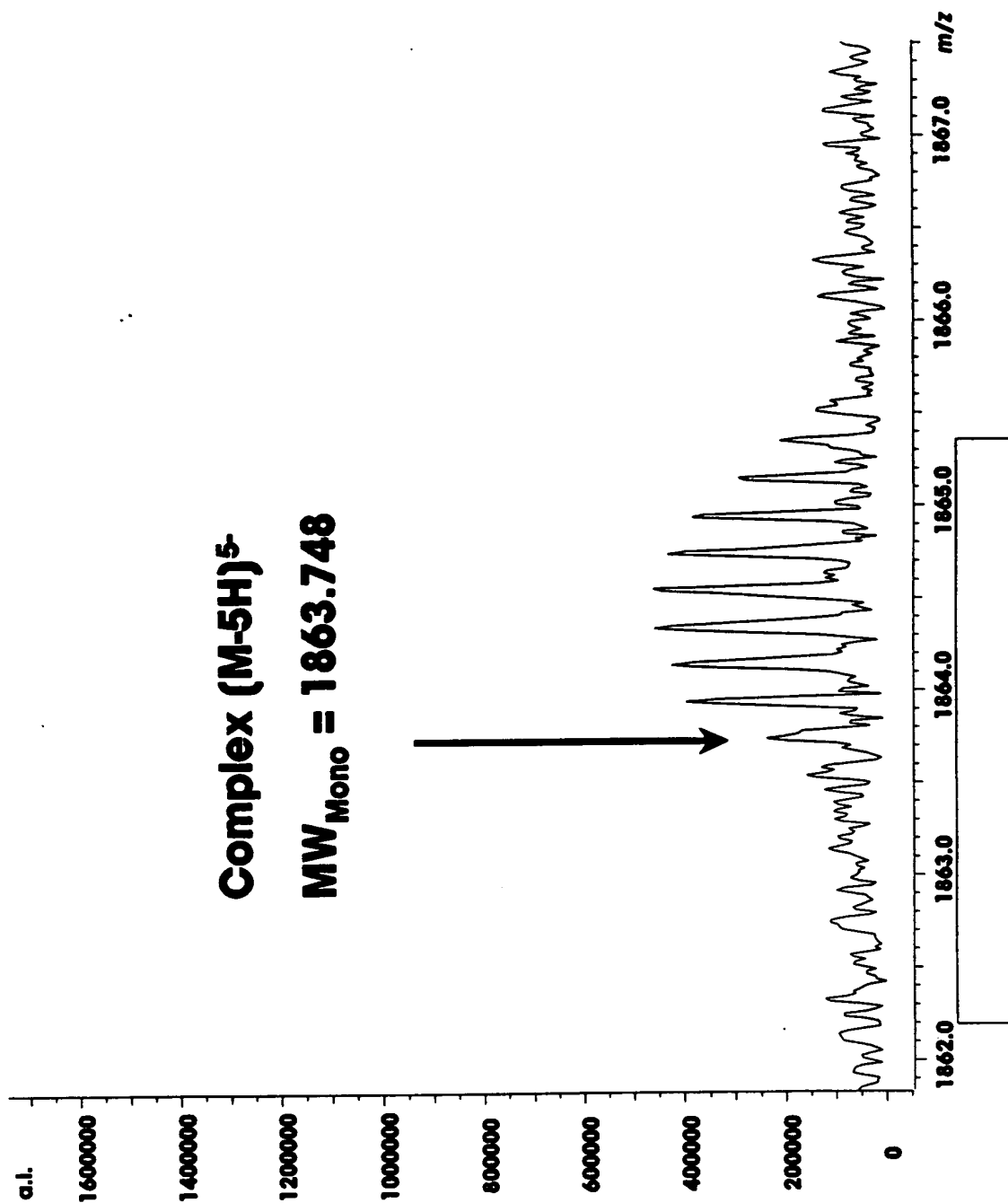


Figure 16

FT-ICR MS of Starting Library

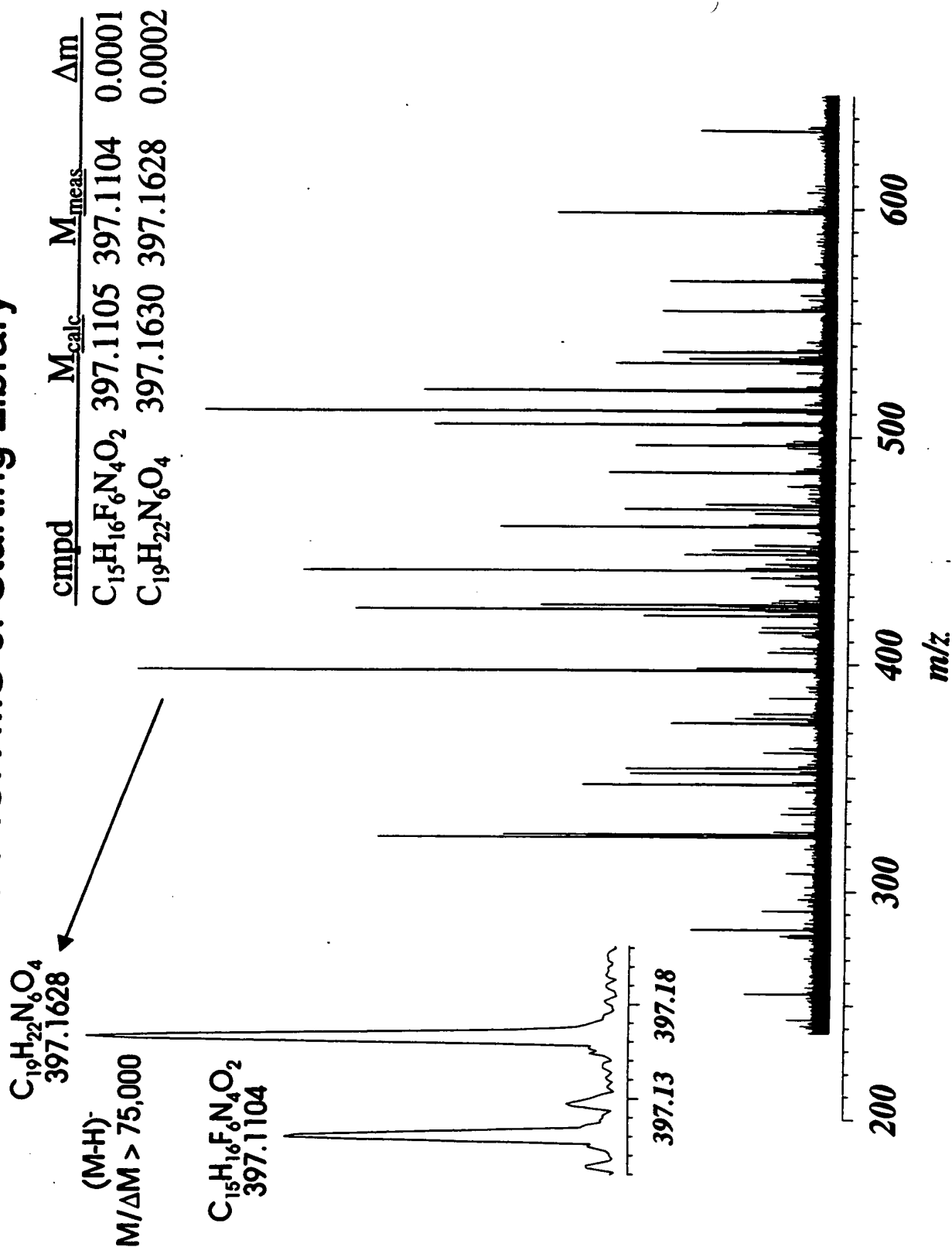


Figure 17
Compound Identification from a 60-member
Combinatorial library with MASS

Complex M_{meas}	9320.300\pm.009 Da
RNA M_{meas}	8922.189\pm.009
ΔM	398.111\pm.009 Da

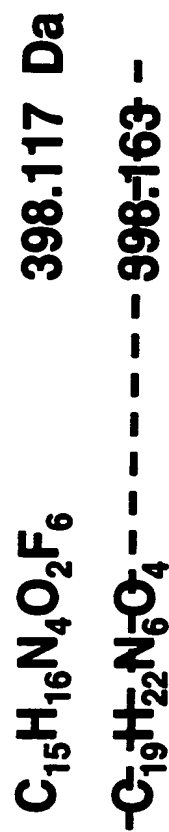


Figure 18

Elemental Composition Constraints

Measured Mass: 615.2969

Mass Tolerance: 1.0 ppm

Charge: 0

Element Min. atoms Max. atoms

¹² C	12	30
¹ H	23	60
¹⁶ O	7	20
¹⁴ N	3	20

Possible Elemental Compositions:

Calc. Mass (amu)	Error (ppm)	Molecular Formula
---------------------	----------------	-------------------

615.296291	0.98	¹⁶ O ₄ ¹⁴ N ₁₉ ¹² C ₂₁ ¹ H ₃₃
615.296298	0.98	¹⁶ O ₉ ¹⁴ N ₁₂ ¹² C ₂₂ ¹ H ₃₉
615.296305	0.97	¹⁶ O ₁₄ ¹⁴ N ₅ ¹² C ₂₃ ¹ H ₄₅
615.296808	0.15	¹⁶ O ₁₅ ¹⁴ N ₁₇ ¹² C ₈ ¹ H ₄₁
615.296815	0.14	¹⁶ O ₂₀ ¹⁴ N ₁₀ ¹² C ₉ ¹ H ₄₇



Further constrain by
 elemental
 composition of
 "letters"

unintended
 products...

"Scaffold"
 $C_{12}H_{23}N_3O_7$

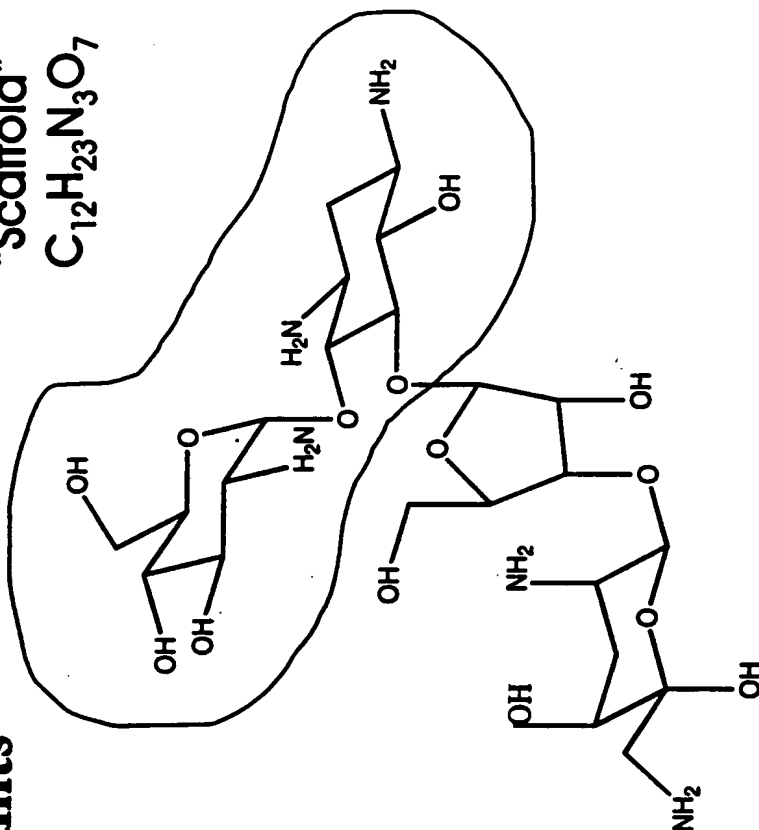


Figure 19

MASS K_d determination for 16S-Paromomycin

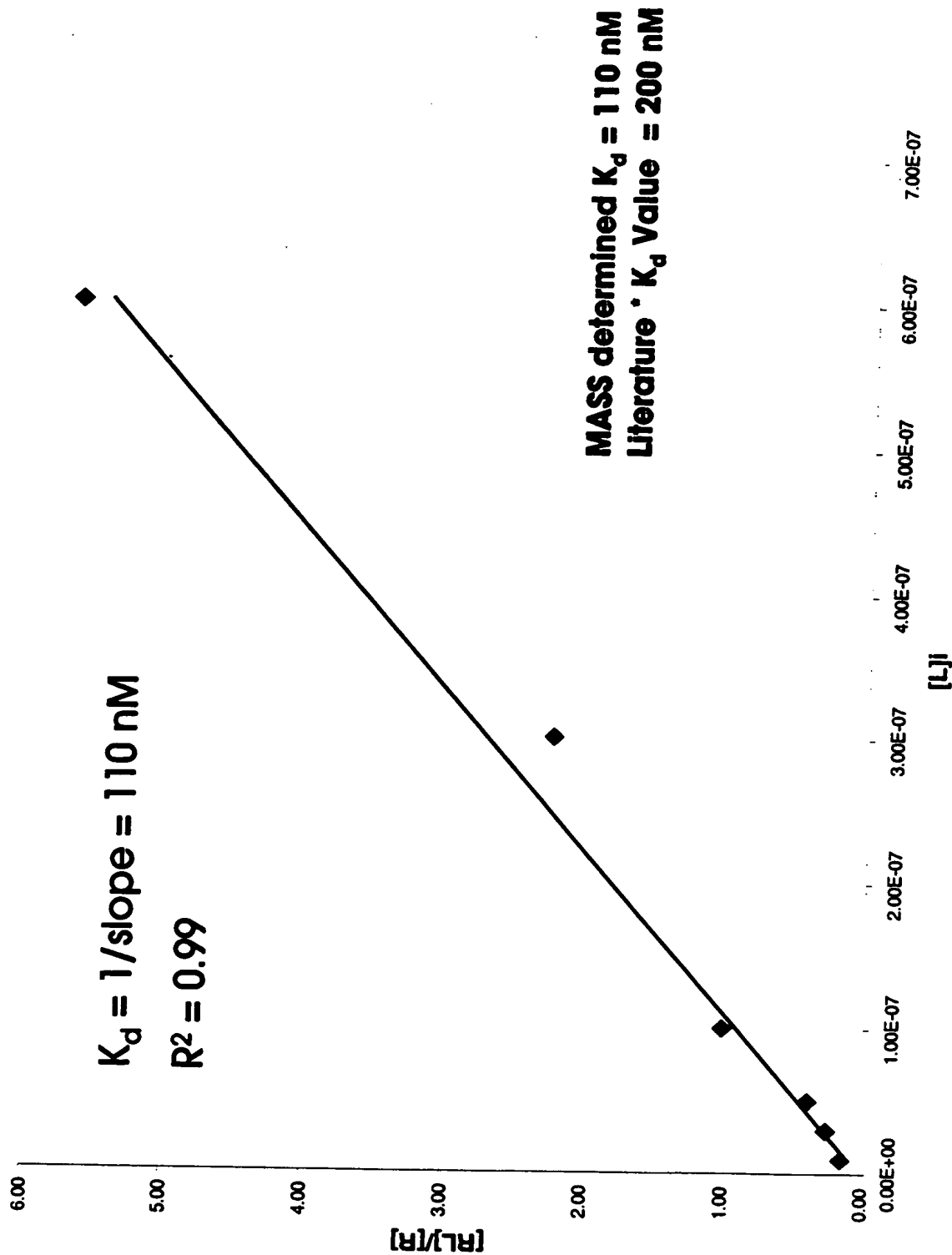


Figure 20 Multitarget Affinity/Specificity Screening

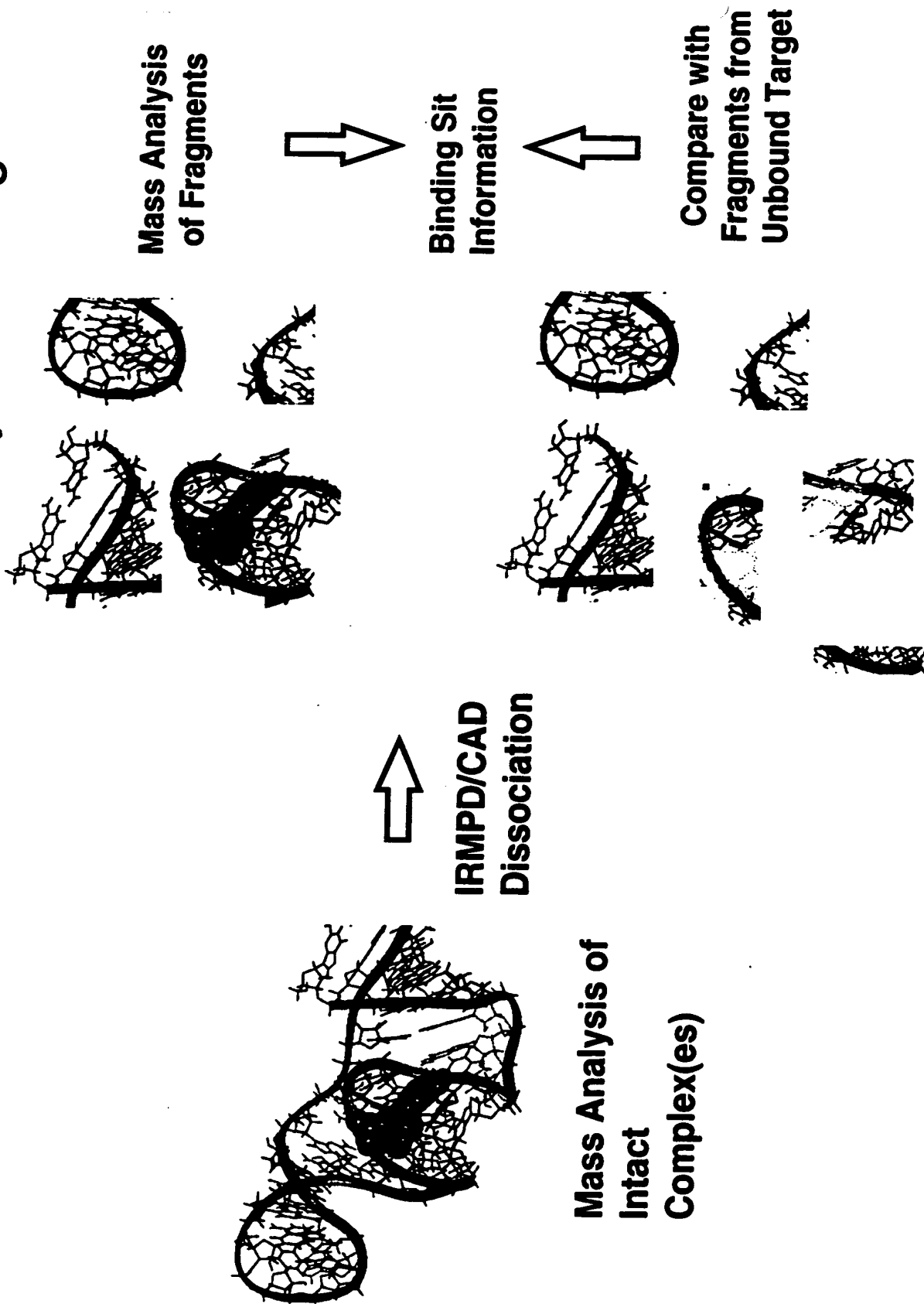


Figure 21

MASS Analysis of 27 Member Library with 16S A-site RNA

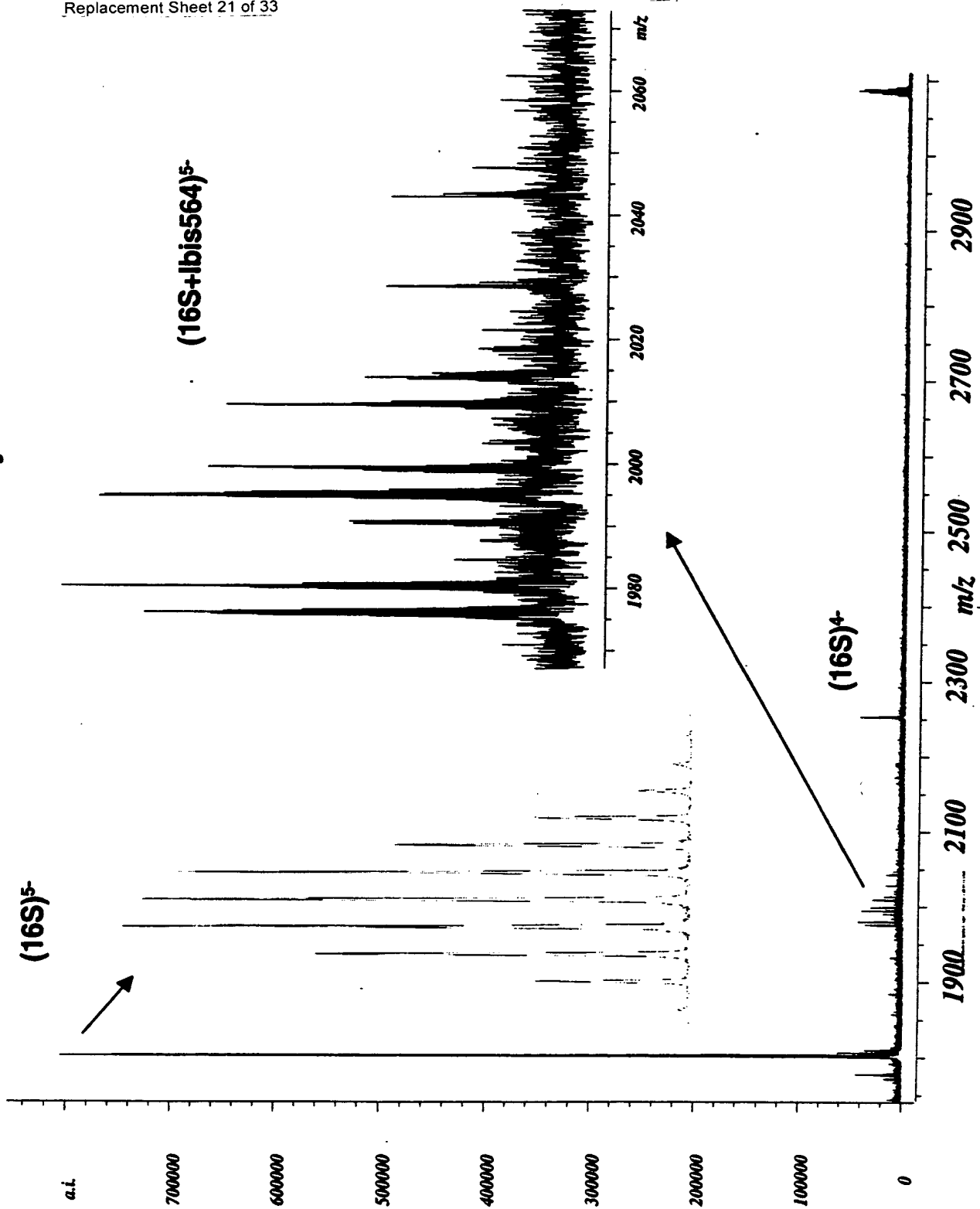


Figure 22

MASS Protection Assay

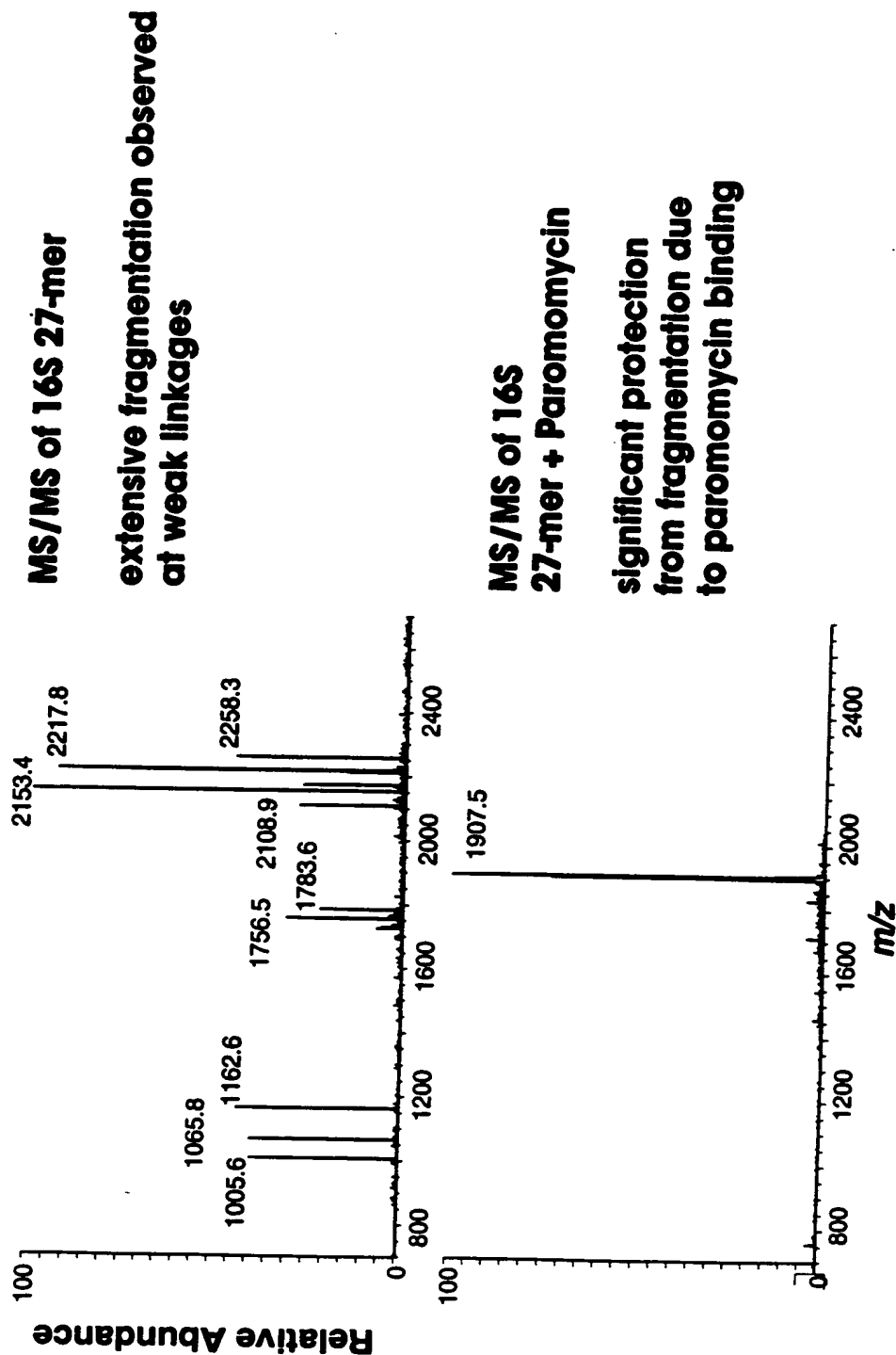


Figure 23 MASS Protection Assay

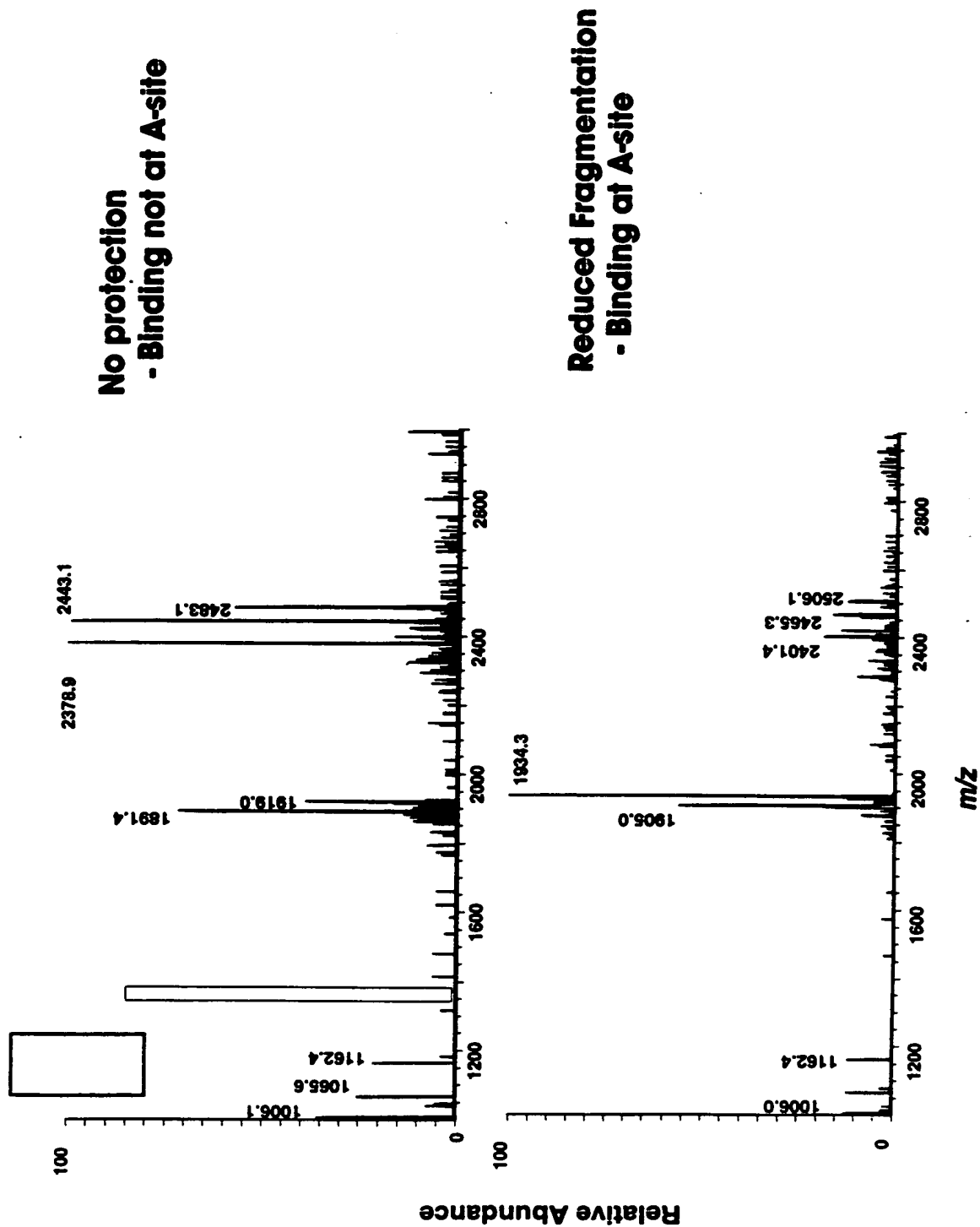
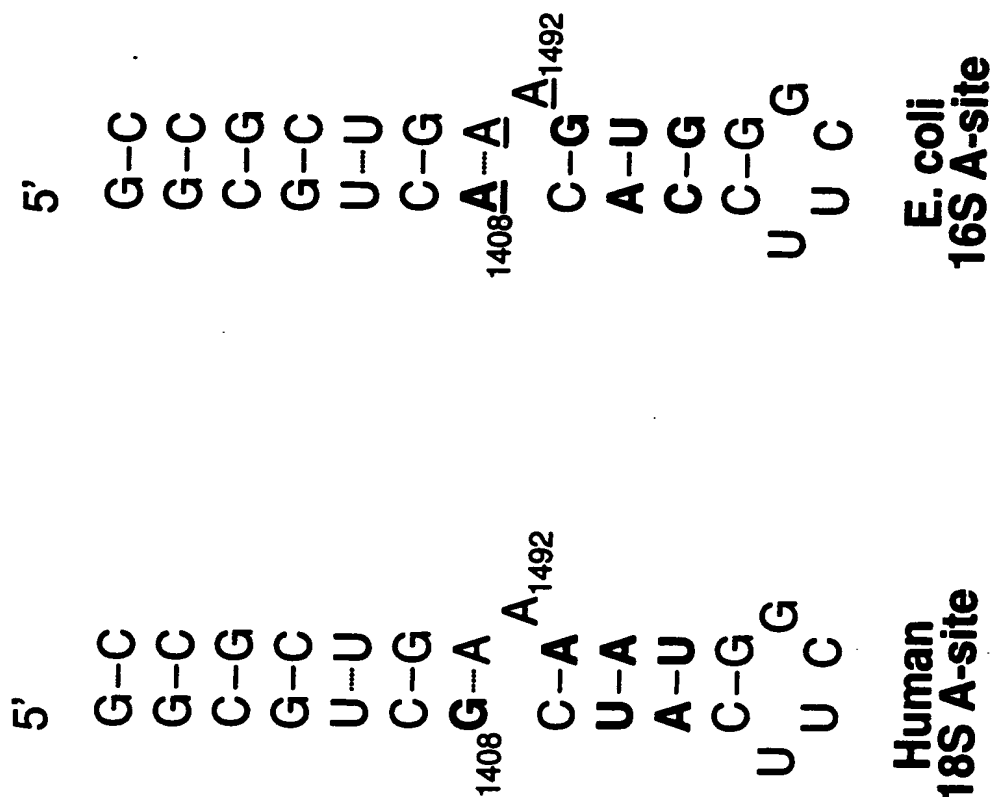


Figure 24

Eukaryotic and Prokaryotic A-Site

Aminoglycoside antibiotics bind to

A-site of decoding region in 16S RNA



Δ MW = 15.011 Da

Figure 25
Neutral Mass Tag Does Not Affect Ligand Binding

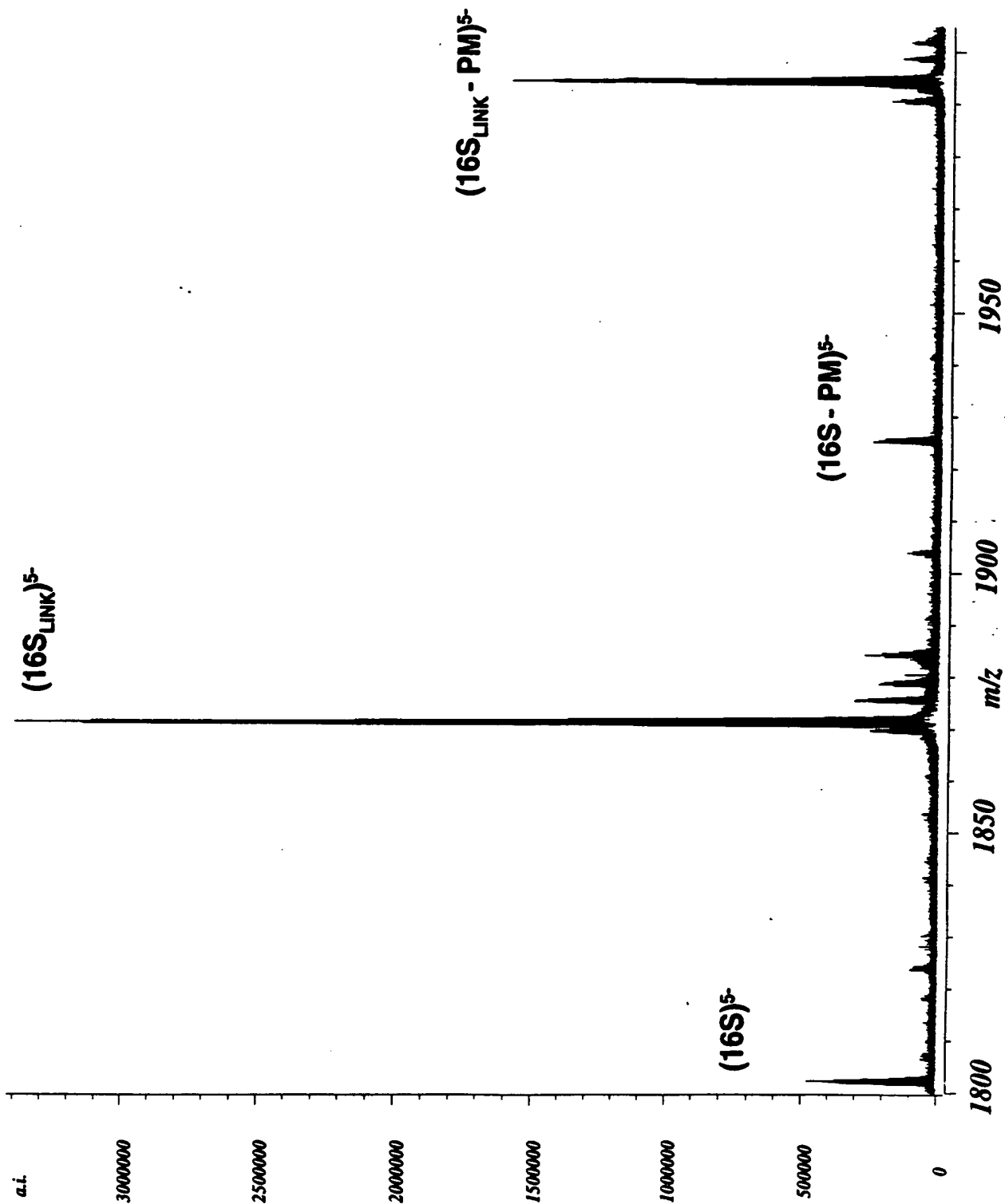


Figure 26

Simultaneous Screening of 16S A-site and 18S A-site Model RNAs Against Aminoglycoside Mixture

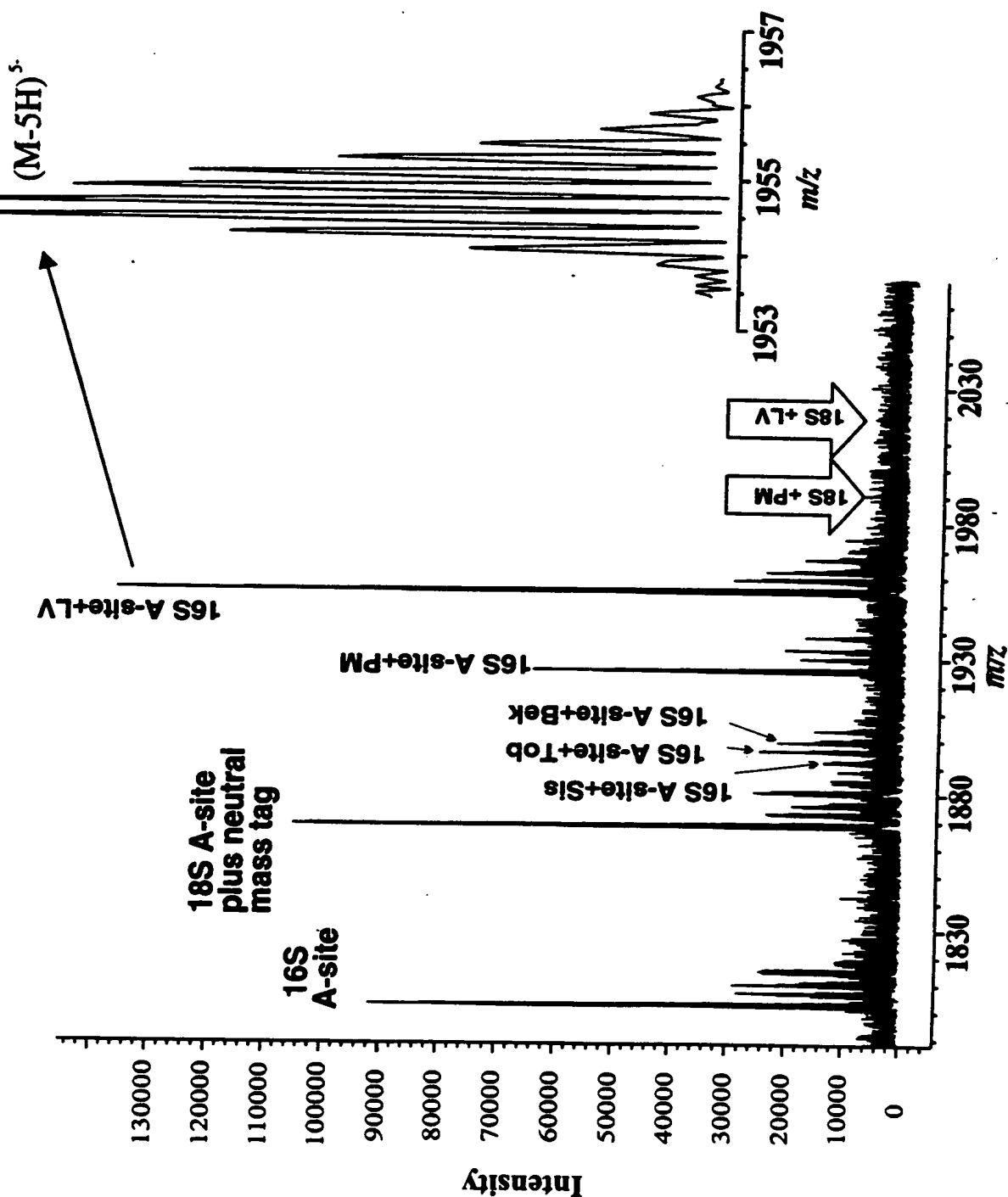


Figure 27

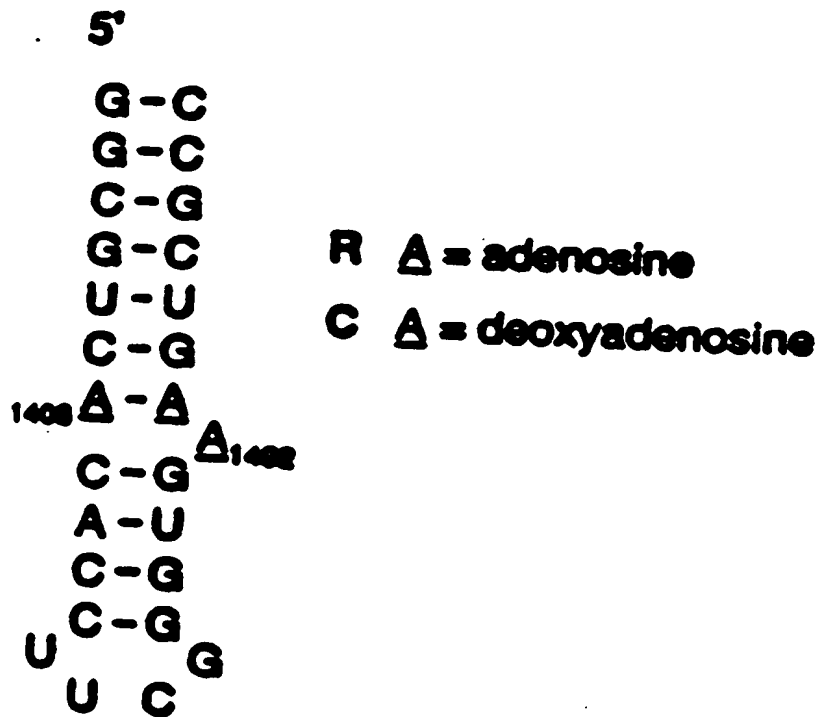


Figure 28

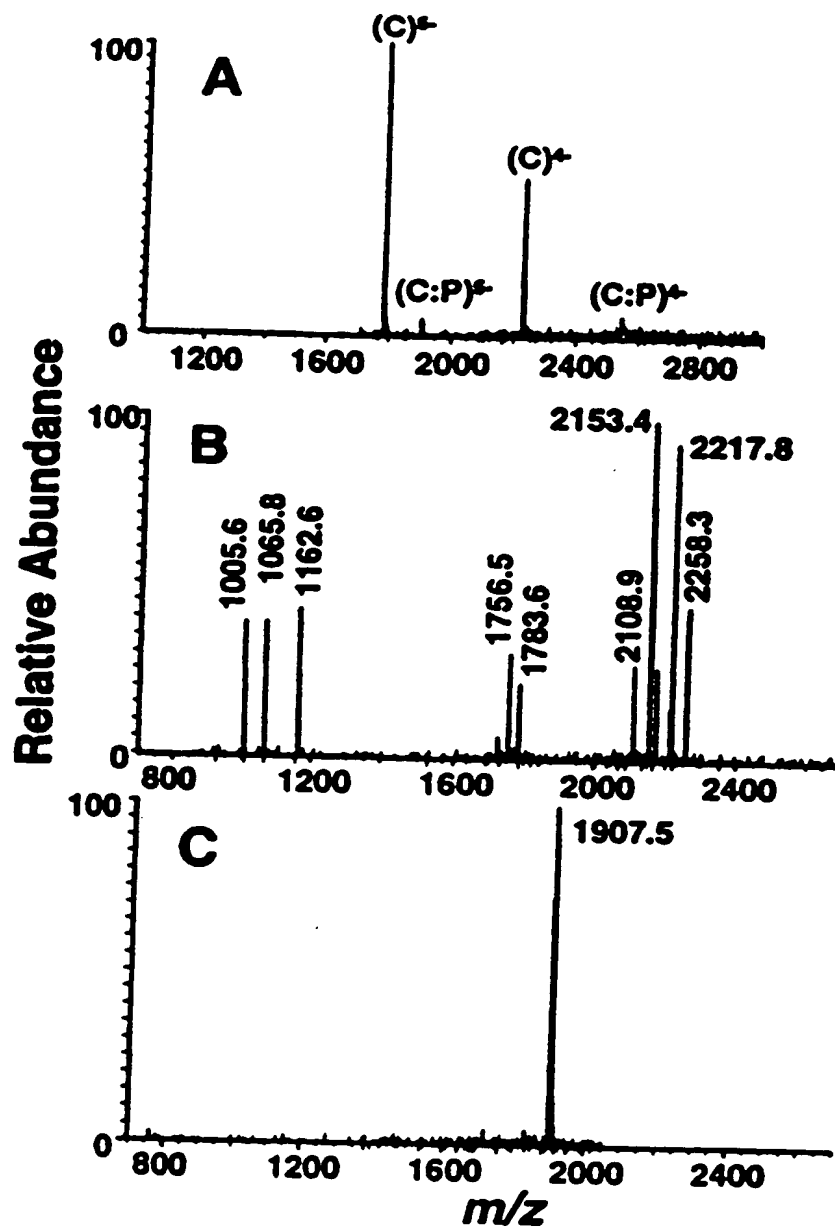
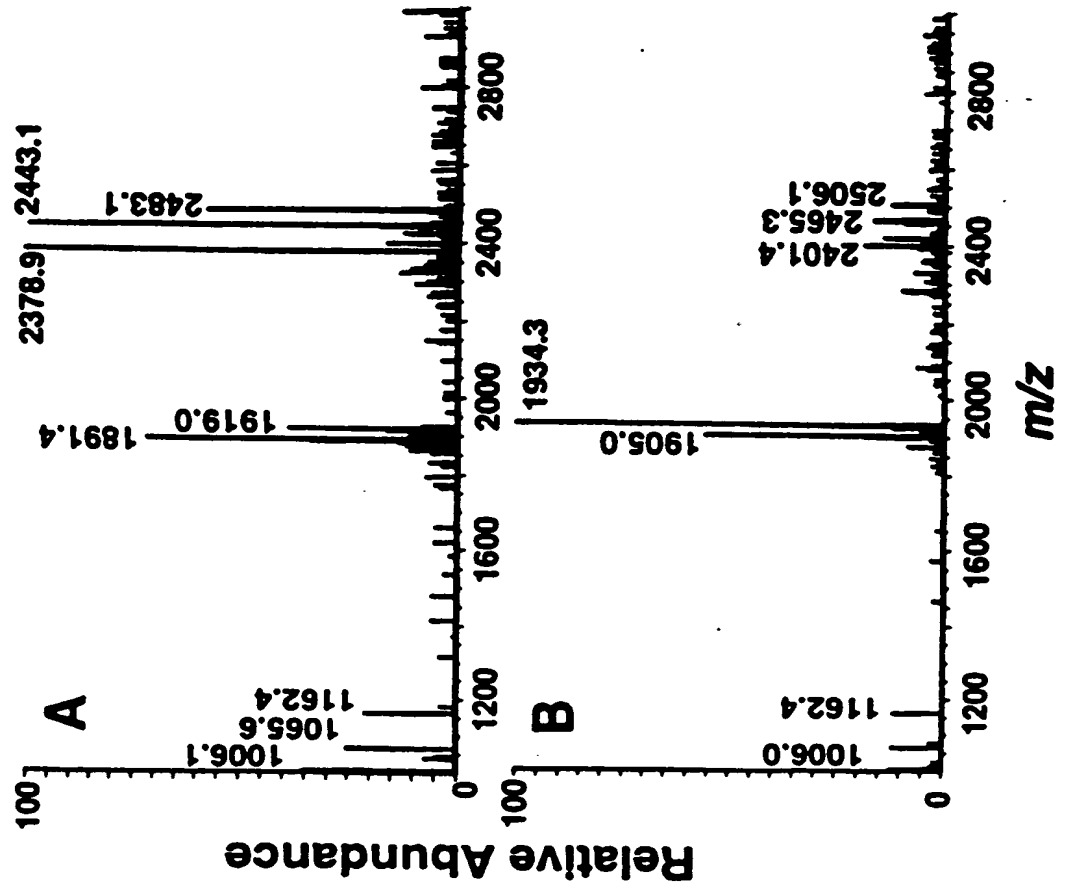


Figure 29



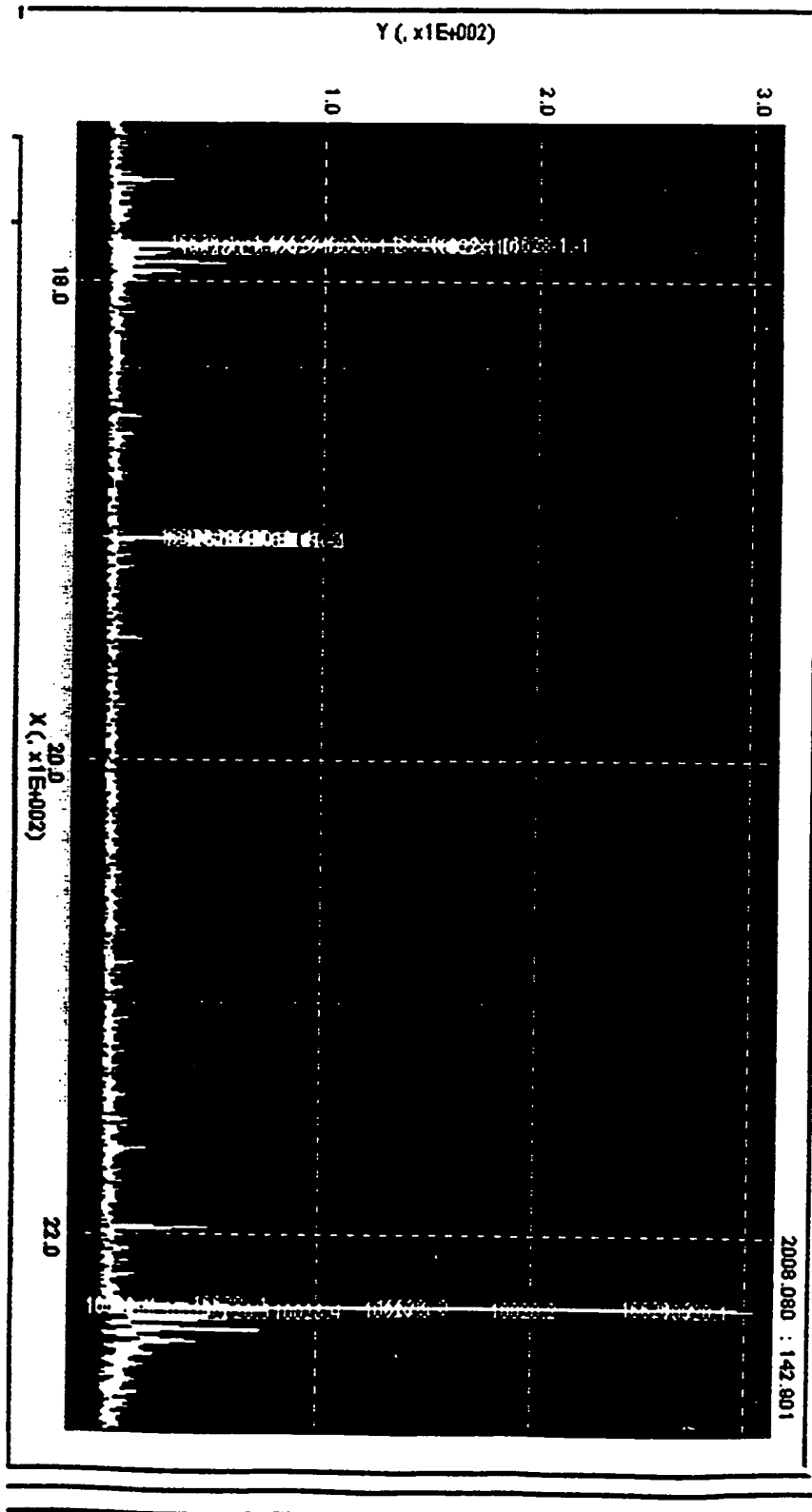


Figure 30

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Figure 31

Figure 32

nr	name	apex	start	stop	height	area
1	16628-1.4	1783.710	1783.635	1783.834	14.55	1.63
2	16628-1.3	1783.909	1783.834	1783.972	60.04	5.15
3	16628-1.2	1784.109	1784.021	1784.184	115.60	11.14
4	16628-1.1	1784.308	1784.233	1784.383	167.34	15.89
5	16628-1.0	1784.508	1784.433	1784.620	133.94	14.74
6	16628-1.1	1784.707	1784.620	1784.795	136.60	13.38
7	16628-1.2	1784.907	1784.795	1784.982	82.63	8.56
8	16628-1.3	1785.107	1785.032	1785.219	57.81	5.21
9	16628-1.4	1785.306	1785.232	1785.369	32.31	2.65
10	16628-1.5	1785.506	1785.456	1785.569	17.67	1.12
11	16628.10019-.4	1906.974	1906.874	1907.031	12.63	1.00
12	16628.10019-.3	1907.173	1907.045	1907.273	22.54	2.11
13	16628.10019-.2	1907.373	1907.287	1907.444	33.86	2.91
14	16628.10019-.1	1907.572	1907.456	1907.701	34.87	3.30
15	16628.10019.0	1907.772	1907.701	1907.843	20.93	1.55
16	16628.10019.1	1907.972	1907.900	1908.043	21.03	1.55
17	16628.10019.2	1908.157	1908.086	1908.271	10.97	0.90
18	16628-.4	2229.874	2229.679	2230.029	27.51	4.87
19	16628-.3	2230.146	2230.029	2230.263	111.72	16.23
20	16628-.2	2230.380	2230.263	2230.516	225.18	32.39
21	16628-.1	2230.633	2230.516	2230.770	280.66	40.90
22	16628.0	2230.887	2230.770	2231.023	287.24	41.95
23	16628.1	2231.140	2231.023	2231.257	242.23	34.17

Graph Table

Figure 33

